Preparation and Characterization of Polyphenol-modified Gelatin Products*

by

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Abstract

We have demonstrated the effectiveness of enzymatic and chemical modification of waste protein from leather, used alone or in combination with protein from the dairy industry, in preparation of fillers for leather treatment. We are continuing to build on these techniques to make products from sustainable resources that can enhance retanned leather. Vegetable tanning, utilizing polyphenols extracted from plant materials, is used primarily for production of heavy leathers for saddles, belts and shoe soles. Recently, the polyphenolic acids involved in vegetable tannage have been investigated extensively for their ability to modify gelatin. We explored whether gelatin, when modified using polyphenols, could give products with properties that might have application in leather processing. In our initial studies, before we investigated individual polyphenolic acids, we tested tannins, e.g. quebracho, to see if indeed these vegetable tannins themselves could be used in crosslinking. These studies demonstrated that gelatin could be modified, using quebracho, to make products that had varying physical properties such as high melting points and viscosities and that molecular weight determinations showed an extensive alteration in gelatin profile. These products were applied to blue stock as fillers and the resultant leather showed improved subjective properties and no significant differences in mechanical properties between treated and control samples. Thus a byproduct (gelatin) from leather-making process, modified with a common polyphenolic tanning agent (quebracho), can be employed to improve crust leather products in the retanning, coloring and fatliquoring process.

Resumen

Hemos demostrado la efectividad en modificar enzimáticamente así como químicamente los rechazos proteínicos del cuero solos o en combinación de proteínas originadas en la industria de lácteos, para la preparación de agentes rellenantes para cuero. Continuamos construyendo en base a estas técnicas para fabricar productos desde recursos sostenibles que pudieran aumentar el rendimiento de cueros recurtidos. El curtido al vegetal, utilizando polifenoles extraídos de plantas, se utiliza principalmente para la producción de cueros pesados para monturas, correas, y suelas pare calzado. Recientemente, los ácidos polifenólicos involucrados en el curtido vegetal han sido investigados extensivamente en cuanto a su capacidad de modificar gelatina. Exploramos si gelatina, modificada por medio de polifenoles, podría dar productos con propiedades que podrían tener aplicación en procesar cuero. En nuestros estudios iniciales, antes de investigar ácidos polifenólicos individualmente, probamos con taninos, v.gr. quebracho, para ver si estos taninos por si mismos servirían como reticulantes. Estos estudios han demostrado que la gelatina puede ser modificada, utilizando quebracho, para producir productos con diversas propiedades físicas tales como aguantar altas temperaturas antes de derretirse y manifestar aumentada viscosidades, y que las determinaciones de los pesos moleculares demostraron una extensa alteración del perfil básico de la gelatina. Estos productos se aplicaron cueros simplemente curtidos al cromo, como rellenantes, y el cuero resultante exhibió aumentadas propiedades sujetivas sin diferencias significantes en las propiedades mecánicas entre las muestras tratadas y sus controles.

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INTRODUCTION

The effectiveness of chemoenzymatic treatment of waste protein from leather, used alone or in combination with protein from the dairy industry, in the preparation of fillers for leather treatment has been demonstrated.[1-8] Further investigations intend to build on these and additional chemoenzymatic methods to make products that can enhance finished leather. For example, such products could be applied to improve veiny hides, a continuing problem in the leather industry that result in lower quality finished leather. The utilization of renewable resources, such as, but not limited to, proteins (e.g. from leather or dairy industries) and carbohydrates (e.g. starch, pectin, and /or chitosan) could be employed. These products could provide better dye uptake or more efficient utilization of fatliquoring agents. They could be used as encapsulating agents to improve the delivery of leather chemicals with less waste, as emulsions in finishing stages of leather processing, as films or coatings for leather finishing, and as possible novel filling agents.

Vegetable tanning, using polyphenols extracted from plant materials, has been employed primarily for the production of heavy leathers used in saddles, belts and shoe soles.[9-10] Polyphenols involved in vegetable tannage have recently been investigated for their ability to crosslink gelatin.[11-16] Polyphenols are known to react under oxidizing conditions with side chain amino groups of peptides, leading to formation of cross-links in proteins.[11] Strauss and Gibson[11] and Mathew and Abraham[17] have demonstrated that biopolymer products could be made by reaction of polyphenols with gelatin and pectins as well as with starch and chitosan. Strauss and Gibson[11] also demonstrated that plant-derived phenolic acids that have been used to cross-link gelatin–pectin coacervates could result in microparticles for use as food ingredients. The authors have also reported that these gels had greater mechanical strength, reduced swelling, and fewer free amino groups. Ou, et al.[18] prepared films from soy protein that had been crosslinked with oxidized polyphenols resulting in films with higher tensile strength. Jones, et al.[19] prepared and characterized biopolymer particles based on thermal treatment of protein (ß-lactoglobulin)-polysaccharide electrostatic complexes and they speculated that these products could be used in encapsulation. Zhang, et al.[20,21] demonstrated that gelatin could be reacted with tannic acid, at pH 8.0 and 58°C resulting in a cross-linked structure that was stable even under boiling, a rigid protein matrix, and at the same time the mechanical properties of films were enhanced. Furthermore when gelatin was reacted with caffeic acid and tannic acid, at pH 9.0, insoluble hydrogels were formed. Evidence was obtained using NMR to confirm that the chemical reactions occurring between the reactive sites of the phenolic compounds and the amino groups in gelatin form C-N covalent bonds as cross-linking linkages in gelatin matrix.

Thus, recent literature suggests polyphenols have the potential to react with gelatin, other proteins (from dairy industry) and with carbohydrates to make products, such as fillers, coatings, films, and microparticles, and that their application could possibly contribute to better quality leather and more efficient use of chemicals in leather processing. We will investigate whether gelatin, alone or in combination with these other resources, when modified using polyphenols could give products with properties that might have application in leather processing. Before we explored individual polyphenolic acids, we tested vegetable tannins, to observe if indeed these tannins themselves could be used in crosslinking. In our first study, we explored whether quebracho would be a viable modifier for producing polymerized gelatin products.

Quebracho (IUPAC Name: [3,5-dihydroxy-2-(3,4,5-trihydroxybenzoyl)oxy-6-[(3,4,5-trihydroxybenzoyl) oxymethyl]oxan-4-yl] 3,4,5-trihydroxybenzoate, CAS Number: 18483-17-5 Chemical Formula: $C_{27}H_{24}O_{18}$, MW 636.5 (22) is a vegetable tannin extracted from heartwood of the red quebracho, or quebracho colorado (*Schinopsis lorentzii*), of the family Anacardiaceae and is obtained mainly from forests of the Gran Chaco of Argentina, Paraguay, and Bolivia.[23] It is a condensed tannin, not decomposed by acids and gradually polymerizes becoming the insoluble derivative, phlobaphenes. Its use, in this form, is limited to tannage of sole-leather according to the process known as "hot-pitting". In order to prevent formation of phlobaphanes, the extract is solubilized by heating under pressure with sodium bisulphite (3-8 % on the extract) at 98°C; part of the bonds in tannin are then split. Initially phlobaphanes dissolve, then not only the size of the tannin molecule decreases but changes in the molecule occur which transform it into soluble tannins the main properties of which are rapid penetration into the pelt.[24]

In this present study, we examine the effect that concentration of quebracho and the pH of the reaction, when reacted with gelatin, will have on physical properties (gel strength, melting point and viscosity). Viable products were applied to leather and their ability to fill leather was evaluated. The products' physical properties, molecular weight distribution by SDS-PAGE, SEM and epi-fluorescent microscopy studies of filled blue stock, as well subjective evaluation of RCF leathers, will be presented.

EXPERIMENTAL

Materials

Commercial Type B gelatin from bovine skin, characterized in this laboratory as 175 grams Bloom, was obtained from Fisher (Fairlawn, NJ). Quebracho was obtained from Hermann Oak Leather Company (St. Louis, MO). Chrome-tan stock (shoe upper) was purchased from a local tannery;

area pieces were sampled from this stock. All other chemicals were analytical grade and used as received.

Preparation of quebracho-modified gelatin products

Gelatin (175 Bloom) samples (5 g) were suspended in water (40 ml) and allowed to swell for 2 h at room temperature (25-28°C); they were stored overnight at 4°C. They were placed in a bath at 65°C until dissolved. Control samples to which no tannin was added, were run to monitor changes in physical properties. The pH was adjusted to 4.0-10.0 with 1 N HCl or 1 N NaOH. Tannin (calculated to be 0 to 5% based on weight of total protein) was prepared in 10 ml of water. The tannin solutions were added with stirring to the protein solutions to give final protein concentrations of 10% w/v for gelatin. Aliquots (10 ml) of all the reaction mixtures were added to test tubes for melting point determination and 30-ml aliquot was poured into appropriate containers (39 mm diameter jar) for determining gel strength. The samples were warmed to 45°C in a shaker bath and the reaction was carried out for 4 h. The samples were cooled to room temperature and then chilled for 17 h at 10°C in a constant temperature bath. Physical analyses (gel strength, melting point and viscosity) were run on these samples. Aliquots of the samples were lyophilized and molecular weight distribution was determined. Sodium azide (70 µl of 1% solution) was added to the remaining treatment solution as a preservative and the samples were stored at 4°C.

Application of biopolymer to wet blue stock (area samples)

Wet blue stock for area and epi-fluorescent study (four pieces, two controls and two tests, approximately 1 foot square) each cut sequentially from the butt, belly or neck area, two pieces / drum, ~325g each), were placed in two small Dose drums (Model PFI 300-34, Dose Maschinenbau GmbH, Lichtenau, Germany), washed (400% float based on hide weight) by tumbling for 30 min at 50°C, drained and refloated in sodium bicarbonate (~1% on hide weight in 400% float). The samples were drummed (in Dose drums) at ambient temperature (25-28°C) until the pH stabilized (6.5-7.0). The floats were drained, the control samples set aside, and to the test samples, the tannin product, 10% gelatin (65 g), based on hide weight (650 g), modified with 2% quebracho (1.3 g), based on gelatin weight, was added in a 400% float (2600 ml). The samples were then drummed for 1 h at ambient temperature and then for 4 h at 45°C. The floats were drained and the samples were washed twice for 10 min at 50°C (400% float), drained, patted dry, and stored at 4°C. The tests and controls were retanned, colored and fatliquored (RCF) using an appropriate shoe upper formula as described in prior publications[3,6]. When completed, all samples were vacuum dried (at 60°C for 6 min) and then hung to dry at ambient temperature and humidity. The samples were wetback and put into plastic bags for 2 hours; the bags were removed and the samples were placed on a shelf in the conditioning room at 20°C and 65% relative humidity for at least 3 days before mechanical properties determinations were performed. No finishing operations were done to the hide pieces.

Analyses:

Physical and mechanical properties.

Gel strength, melting point, and viscosity of the quebracho-treated proteinaceous solutions were determined as described in previous publications[25]. Mechanical properties (tensile, elongation, Young's Modulus, toughness index, tear strength, and thickness) were determined as described in a previous paper[26].

Molecular Weight Determination

Protein molecular weights were estimated as described previously[27]. In summary, SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecyl sulfate) was run using precast 4-15 percent gradient gels. A broad range SDS-Standard (BRM) calibration standard (Bio-Rad, Hercules, CA), which contains a mixture of nine proteins ranging in size from 6,500 to 200,000 Daltons, was used. Samples of lyophilized protein were dissolved in sample buffer (10 mM Tris-HCl at pH 8.0 containing 1 mM EDTA, 2.5 percent SDS, 5 percent β-mercaptoethanol and 0.01 percent bromphenol blue) and were then heated at 40°C for 4 hr. Separation was achieved using a Phast-Gel System (Pharmacia Biotech Inc., Piscataway, NJ). Gels were stained with Coomassie Blue (Pharmacia) and were scanned with a Personal Densitometer SI and analyzed using ImageQuaNT v:4.1 software (Molecular Dynamics, Inc., Sunnyvale, CA).

Hydrothermal stability

Hydrothermal stability of quebracho-modified gelatin was determined on a Multi-Cell Differential Scanning Calorimeter (DSC) (model CSC-4100) from Calorimetry Sciences Corporation, Lindon, UT, as previously described.[28] In preparation for DSC experiments, unmodified and modified-gelatin (100-150 mg) samples were weighed into ampoules and a small amount of distilled water (500 µl) was added; the ampoules were sealed and placed in the calorimeter. The calorimeter was programmed to record heat flow as µcal/°C while the temperature was increased from 10°C to 180°C at 1.0°C/min with an equilibration period of 600 s at the start. The temperature at the peak of the calorimetry trace, T_p, was considered to be an apparent melting temperature.

Subjective evaluation RCF leather

Each treated and untreated sample, two samples of each, was evaluated (one evaluator) with respect to handle, fullness, grain (break) and color. A value from 1 to 5 was assigned for each parameter, with 1 being the worst and 5 being the best. From these ratings, an overall evaluation was determined and this value (from 1 to 5) was reported.

Optical microscope equipped with Epi-fluorescent attachment

The treated wet blue samples were sectioned, using a razor (grain to flesh) and mounted onto a glass slide. They were examined using an Eclipse 6600 Polarizing Microscope (Nikon Instruments Company, Melville, NY), at 4X magnification, operating in optical mode. The instrument was equipped with a X-Cite™ 120 Fluorescence Illuminator System which was fitted with a metal halide lamp (EXFO Photonic Solutions, Inc., Mississauga, ON, Canada), with two filter cubes or optical blocks, containing epi-fluorescence interference and absorption filter combinations including an excitation filter, dichromatic beamsplitter (often referred to as a mirror), and a barrier (or emission) filter (515-555nm or 600-660nm)[29], and with a digital camera (DXM 1200).

Scanning Electron Microscopy (SEM)

Wet blue samples, after treatment and after RCF, along with their respective control samples were cut into small strips (6.5 cm × 1 cm), placed in a test tube to which nano pure water was added (to cover strip) and freeze-dried. Two pieces (1.5 mm) were cut from each of the dry samples and were mounted onto the surfaces of carbon adhesive tabs with the help of Duco cement. After drying for at least 1 h, silver paint was applied to the exposed surface area around the samples. The samples were sputter-coated with a thin layer of gold using a Scancoat Six Sputter coater. Samples were viewed using a Quanta 200 FEG Environmental Scanning Electron microscope, FEI Company (Hillsboro, OR) in high vacuum-secondary electron imaging mode at an accelerating voltage of 10 kV (spot size 3.0, pressure 0.3 torr). Digital images were collected at 50, 250, 500, and 1000× magnification.

RESULTS AND DISCUSSION

Preparation of quebracho-modified gelatin

It has been suggested in the literature that polyphenolic acids have the capability of modifying gelatin.[11-21] It has also been reported that some vegetable tannins themselves could be applied to gelatin to give products with interesting physical properties.[30] Prior to investigating individual polyphenolic acids, we examined the effect, if any, that vegetable tannins, specifically quebracho, would have on properties of gelatin. After determining that the solubility of quebracho (sulphited) in water (up to 10%) would not be a problem, we subjected 10% w/v concentrations of gelatin to reaction with varying concentrations of quebracho at varying pHs.

Type B gelatin (pH 5.0-7.5) when used in our previous studies was reacted with various cross-linking agents at pH 6.0 to 7.0. In our initial experiment, we treated gelatin in this pH range with 0 to 2.0 % quebracho at a temperature of 45°C for 4 h. We found (Figure 1a, b, c) that the quebracho, at concentrations between 0-2%, had no significant effect on the gelatin's

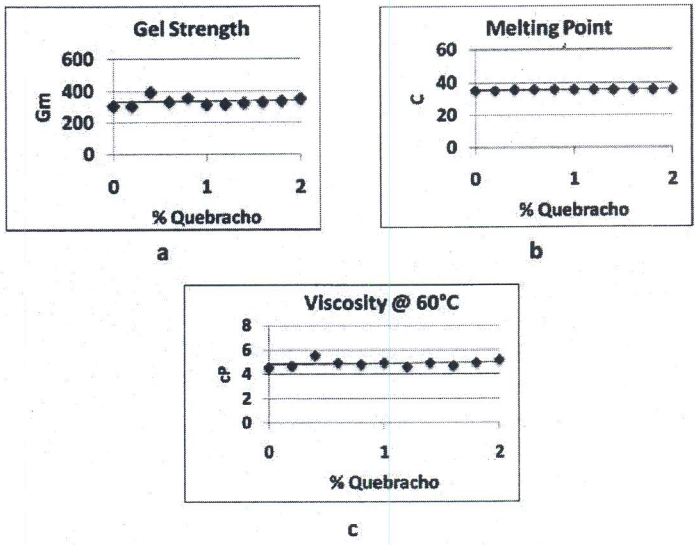


Figure 1. 175 Bloom gelatin (10% w/v) treated with 0-2.0% quebracho @ pH 6.5-7.0, 45°C for 4 h (a) gel strength; (b) melting point; (c) viscosity @ 60°C.

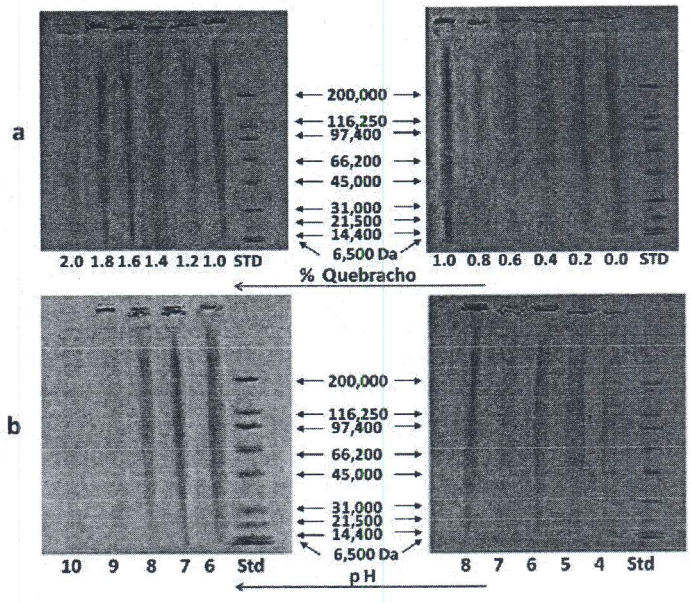


Figure 2. SDS-PAGE of 175 Bloom gelatin, 10% w/v concentration, treated with 0-2% quebracho at pH 6.5-7.0, (a) and with 2% Quebracho @ pH 4-10, 45°C for 4 h (b); molecular weights are shown in Da.

physical properties (gel strength, melting point and viscosity); furthermore, no changes were discernible in the molecular weight distributions (Figure 2a).

In the next series of experiments, we adjusted the pH of the reaction from 4-10 while keeping the quebracho concentration constant at 2.0%. When the reaction was run from pH 4.0 to 8.0, no change was apparent in either physical properties (Figure 3a, b, c, test) or SDS-PAGE gels (Figure 2b). However when the reaction was run from pH 8 to 10.0, there was a significant change in both the physical properties (Figure 3a,

b, c, test) and molecular weight distribution (Figure 2b). The gel strength decreased from 331 to 284 g, a phenomenon we have seen when more cross-links are introduced into the gelatin. From pH 8.0 to 9.0, the melting point increased from 36.4 to 54.2°C and from pH 9.5 to 10.0, the product did not melt. The viscosity did not change significantly from 8.0 to 9.0 but at pH 9.5 to 10.0 it could no longer be read at 60°C. The molecular weight distribution (Figure 2b) showed that the bands indicative of gelatin had grown less intense at pH 8.0, at pH 9.0, only the band that typically does not enter the gel remained, and at pH 10.0, all bands had disappeared

Next we examined the effect of quebracho concentration on reaction with gelatin. From 0 to 5 % quebracho was added to 10% w/v concentration of gelatin and these samples were heated at 45°C for 4h at pH 9.0. As the concentration of quebracho increased, the physical properties of the products changed (Figure 4a, b, c).

The gel strength increased slightly from 322 to 335 g, while the melting point increased dramatically from 37.2°C at the 0% concentration to 60.0°C at the 2% concentration; the higher concentrations (3, 4, and 5%) did not melt. The viscosity could only be read for the 0, 1, and-2% quebracho concentrations and these ranged from 4.61 to 5.82 cP@60°C. When one examines the molecular weight distribution (Figure 5a), the bands indicative of gelatin (from 6500 to over 200, 000 Da) and the high molecular weight band that typically does not enter the gel, have basically disappeared when one reaches the 5% quebracho concentration.

To insure that neither the quebracho alone, at varying concentrations at pH 9.0, nor gelatin alone at pHs from 4-10, were influencing the changes in physical properties that we observed, both of these parameters were run under these specified conditions. As the concentration of quebracho was increased from 0 to 5%, there were no observable changes in viscosity (varied from 0.62 to 0.65 cP @ 60°C) The physical properties of the gelatin (Figure 3a, b, c, control) changed slightly depending on the pH and the data shown correlates with that which was found in an earlier study.[31] The molecular weight distribution study for gelatin (Figure 5b) is corroborating these results. The data are showing that the quebracho and gelatin alone are not contributing to the significant changes in physical properties that we are observing at the higher concentration and pH range.

Based on the above data, we selected the 10% gelatin/2% quebracho product prepared at pH 9.0, 45°C, for 4 h, to be utilized in filler studies. The physical properties of the product are shown in Table 1 and the standard deviations listed are typical of data from multiple replicates found throughout this series of experiments and indicate that the reaction is quite reproducible; the properties of unmodified gelatin are shown for comparison.

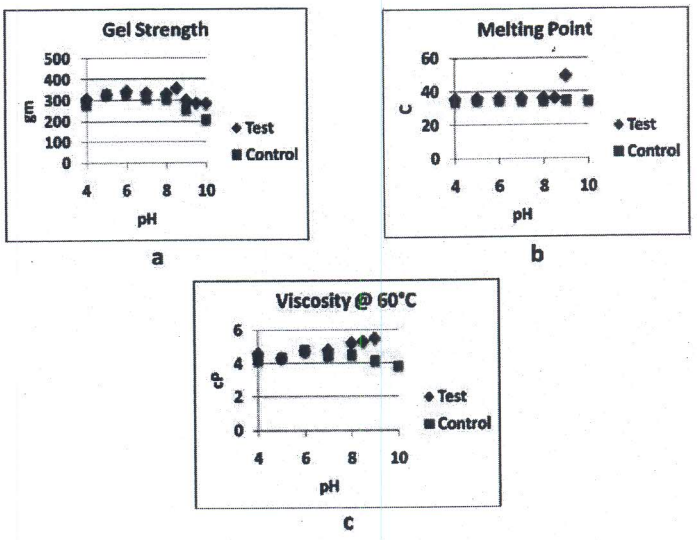


Figure 3. 175 Bloom gelatin (10% w/v) treated with 0% (control) and 2% quebracho (test) @ pH 4-10, 45°C for 4h; (a) gel strength; (b) melting point; (c) viscosity @ 60°C.

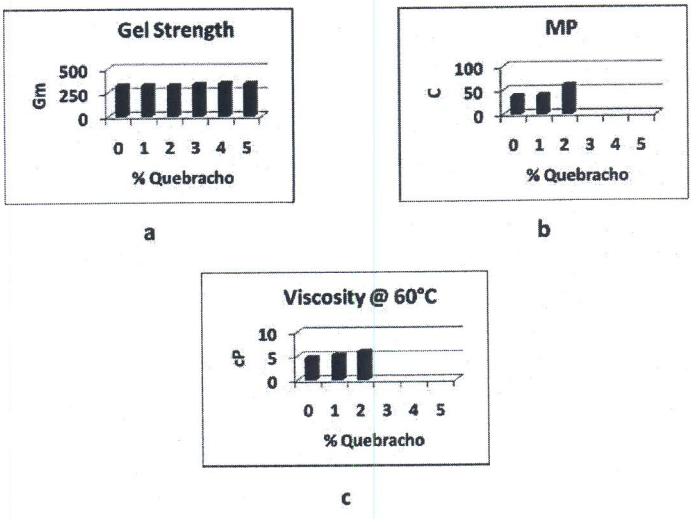


Figure 4. 175 Bloom gelatin (10% w/v) treated with 0-5% quebracho @ pH 9.0, 45°C for 4h; (a) gel strength; (b) melting point; (c) viscosity @ 60°C.

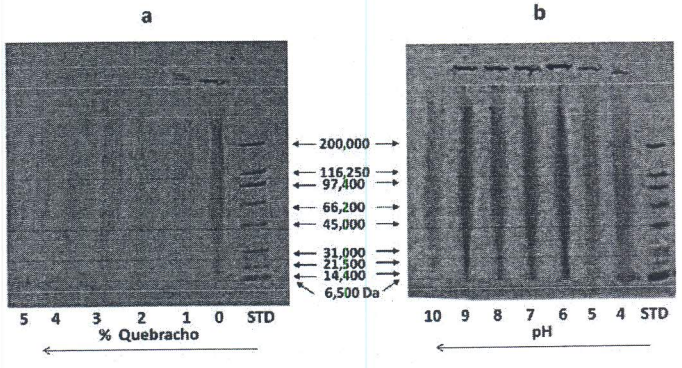


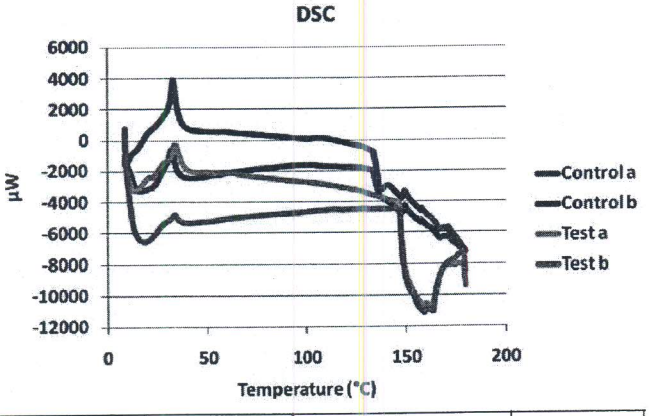
Figure 5. SDS-PAGE of 175 Bloom gelatin (10% w/v) treated with 0-5% quebracho @ pH 9.0 (a), and with 0% quebracho, pH 4-10 (b) at 45°C for 4h; molecular weights are shown in Da.

TABLE 1
Physical Properties of Quebracho-modified Gelatin

Product	Gel Strength (g)	MP (°C)	Viscosity @ 60°C (cP)
10% Gelatin/ 0% Quebracho[ab]	315.6	36.1	4.58
Std Dev	6.6	1.1	0.03
10% Gelatin/ 2% Quebracho[ac]	321.0	51.8	5.47
Std Dev	15.0	7.9	0.20

[a]pH 9.0 @45°C for 4 h; [b]N=2; [c]N=6

The thermal stability of the product was further examined by DSC and the results are shown in Figure 6. The data show that in the unmodified gelatin sample (Control a and b), there is an apparent melting (endothermic) at about 30.7°C and after approximately 133°C the sample appears to decompose. In the modified sample (Test a and b), there is a peak at about 33.9°C (endothermic), indicating melting, but a new peak (exothermic) appears at about 159.5°C and possibly is indicating the "plasticized protein" type product as described in the literature.[21]



Sample	Endothermic Peak (°C)	Exothermic Peak (°C)
10% Gelatin/0% quebracho (Control)	30.7	-
10% Gelatin/2% quebracho (Test)	33.9	159.5

Figure 6. DSC analysis of 175 Bloom gelatin (10% w/v) treated with 0% quebracho at pH 9 (control a and b), and with 2% quebracho @ pH 9 (test a and b), 45°C for 4h; table indicates melting (endothermic) and exothermic temperature peaks.

Evaluating filling capability of products

Epi-fluorescent microscopy and SEM study of blue stock

In a previous study[2] we attached fluorescent labels to protein samples that were to be applied to the hides as fillers and thus we could determine if these products were evenly distributed throughout the hides and were not removed by washing. In this present study, prior to labeling we checked to see if the gelatin-quebracho product itself fluoresced. Subsequently samples of unmodified 10% w/v gelatin and gelatin modified with 2% quebracho were prepared and then checked for fluorescence using an epi-fluorescent microscope. The unmodified gelatin fluoresces only slightly (Figure 7a) whereas the quebracho-modified gelatin fluoresces strongly (Figure 7b).

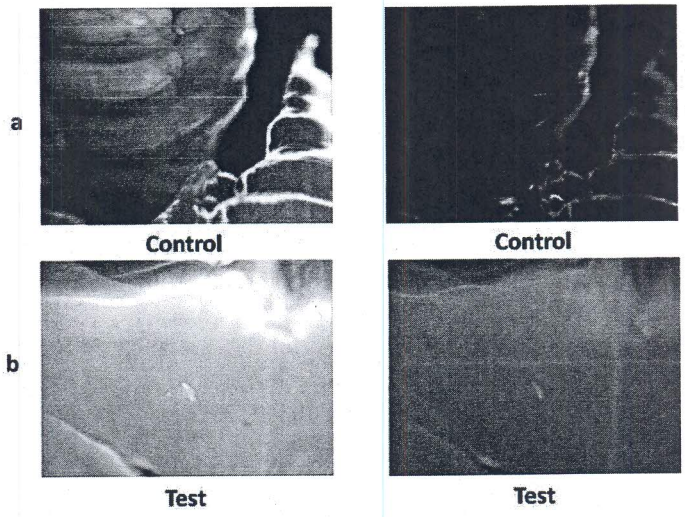


Figure 7. Epi-fluorescent micrographs of untreated gelatin (a) and quebracho treated gelatin (b); 10% (w/v) 175 Bloom gelatin samples were incubated with 0 and 2% quebracho @ pH 9.0, 45°C for 4h; two emission (barrier) filters (between 515-555nm and 600-660nm) were used.

Pieces of blue stock were treated with the gelatin-quebracho product (10% gelatin/2% quebracho @pH 9.0, 45°C for 4 h) as described in experimental section. After washing, small samples were taken and inspected using an epi-fluorescent microscope. The treated wet blue product was examined, and when compared to untreated control samples, showed that the leather was filled (Figure 8a and b) and the images indicated that the filler was not removed during the two washings.

The wet blue was also examined using SEM. Differences in structure between tests and controls in blue stock can be seen and representative images (1000x) are shown (Figure 9a). The fiber structure of the untreated sample (control) appeared more distinct whereas the fiber structure in the gelatin/ quebracho treated blue stock (test) is not resolved.

Analysis of RCF (SEM and subjective and mechanical properties)

The treated blue stock and the untreated control samples were RCF, using a formula described previously.[3,6] The RCF stock was examined by SEM and differences in fiber structure, similar to those we observed in the blue stock, can be seen in the test samples and the controls (Figure 9b). When the samples were

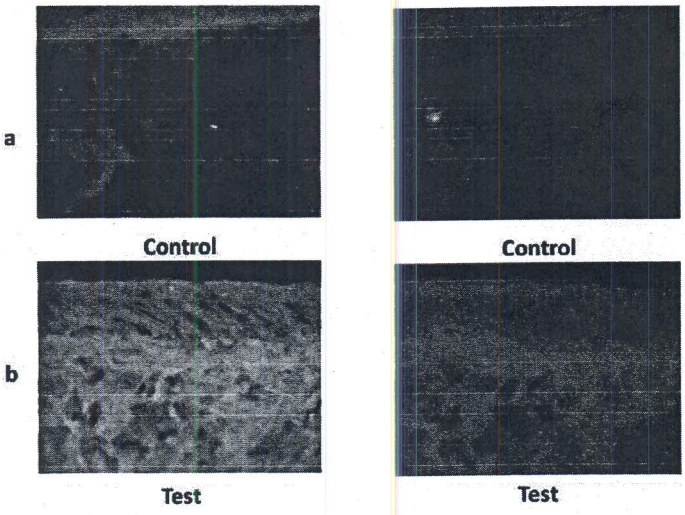


Figure 8. Epi-fluorescent micrographs of blue stock; (a) untreated control sample and (b) test sample treated with quebracho modified gelatin (10% gelatin, 2% quebracho, @ pH 9.0, 45°C for 4 h; two emission (barrier) filters (between 515-555nm and 600-660nm) were used.

examined at 1000X magnification, the control samples show distinct bundles of fibers. However in the test samples, these bundles are not apparent. In previous studies,[2,3] SEM images of filled samples showed distinct differences from untreated samples when blue stock was examined, but we rarely saw differences in the crust such as we are seeing in this study.

The crust was subjectively evaluated with respect to handle, fullness, break, color and overall on a scale of 1 to 5, with 1 being the worst and 5 being the best. Two samples each of controls and tests were run and evaluated in each trial. Thus the ratings (from one evaluator and averaged from 3 trials for a total of six evaluations for each parameter) can be seen in Figure 10. In every parameter the gelatin-quebracho treated leather samples were significantly improved over the untreated control samples. Thus, gelatin-quebracho products have the potential to impart additional characteristic properties to the leather which could result in a higher economic return due to improved quality and the potential increase in cutting area.

Mechanical properties (thickness, tensile strength, elongation, Young's Modulus, toughness index, and tear strength) were determined on the treated crust leather along with the untreated control samples. The data from each property for the eight samples (two trials) examined, four control samples and four test samples, were averaged (Figure 11). The results show that there are no significant differences, as indicated by the error bars, between the test and the control samples in any of the properties examined.

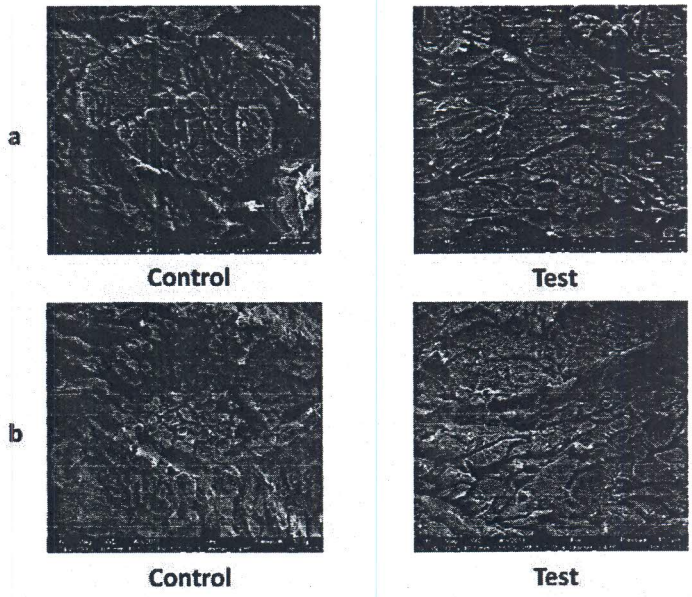


Figure 9. SEM micrographs (1000x) of untreated control samples, and test samples treated with 175 Bloom gelatin (10% w/v) modified with 2% quebracho @ pH 9, 45°C for 4 h, (a = wet blue; b= crust); (— = 20μm).

CONCLUSIONS

When gelatin is reacted with the vegetable tannin quebracho, at pH 9.0-10.0, changes in the physical properties are observed. Gel strength has not been affected significantly, but there is a significant increase in melting point and viscosity. Molecular weight distribution studies correlate with the physical property data. Quebracho-treated gelatin has fluorescent properties and when samples of the treated blue stock were examined using epi-fluorescence microscope and compared to an untreated control sample, fluorescence was indicating that the blue stock was filled and that the filler was not removed by washing. With respect to SEM analysis, distinct differences in fiber structure between treated and untreated samples can be seen. Samples of treated blue stock along with controls were RCF and were subjectively evaluated. In every subjective property evaluated, there was an improvement in the treated samples. Mechanical properties indicated that there were no significant differences between tests and controls. Thus a byproduct (gelatin) from leather-making process, modified with a common polyphenolic tanning agent (quebracho), can be employed to improve crust leather products in the retanning, coloring and fatliquoring process.

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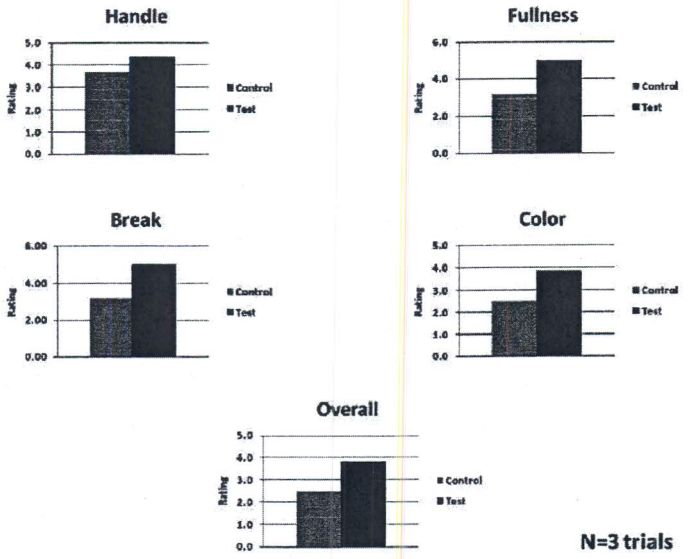

Figure 10. Subjective evaluation (handle, fullness, break, and overall) using rating scale of 1 = worst to 5 = best, of wet blue (area pieces), treated with pH-adjusting agents alone (controls) and with quebracho-modified gelatin (tests) @ pH 6.5-7.0, 45°C for 4 h, then RCF; data are averaged from three trials.

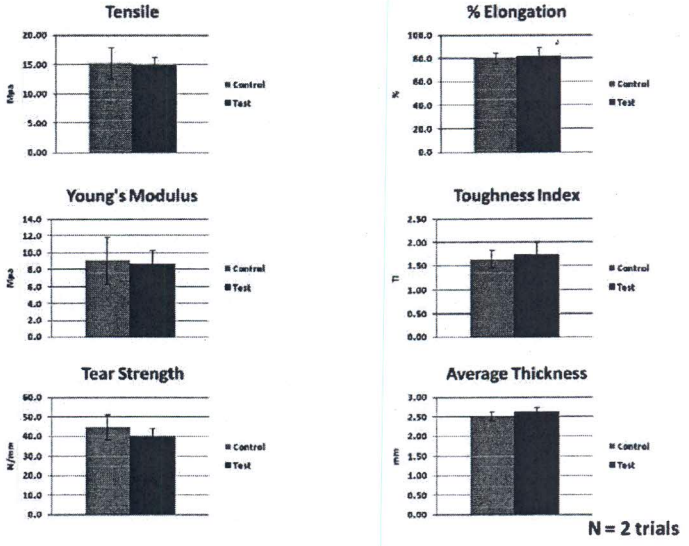

Figure 11. Mechanical properties (with STD Dev) of area pieces of wet blue, treated with pH-adjusting agents alone (controls) and with quebracho-modified gelatin (tests), @ pH 6.5-7.0, 45°C for 4 h then RCF; data are averaged from two trials.

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